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REVIEW ARTICLE

Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities

Yankai Wen[1], Joeri Lambrecht[2], Cynthia Ju[1] and Frank Tacke[2]

Macrophages, which are key cellular components of the liver, have emerged as essential players in the maintenance of hepatic homeostasis and in injury and repair processes in acute and chronic liver diseases. Upon liver injury, resident Kupffer cells (KCs) sense disturbances in homeostasis, interact with hepatic cell populations and release chemokines to recruit circulating leukocytes, including monocytes, which subsequently differentiate into monocyte-derived macrophages (MoMϕs) in the liver. Both KCs and MoMϕs contribute to both the progression and resolution of tissue inflammation and injury in various liver diseases. The diversity of hepatic macrophage subsets and their plasticity explain their different functional responses in distinct liver diseases. In this review, we highlight novel findings regarding the origins and functions of hepatic macrophages and discuss the potential of targeting macrophages as a therapeutic strategy for liver disease.

Keywords: Kupffer cells; monocyte-derived macrophages; liver inflammation; liver fibrosis; liver cancer

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INTRODUCTION

Macrophages, the most abundant liver immune cells, play a critical role in maintaining hepatic homeostasis and the underlying mechanisms of liver diseases.[1] Hepatic macrophages consist of resident macrophages, Kupffer cells (KCs), and monocyte-derived macrophages (MoMϕs). Our previous review article published in 2016 highlighted research elucidating the heterogeneity of hepatic macrophages and the involvement of different subsets of macrophages in pathophysiological conditions of the liver. We also discussed potential strategies for targeting hepatic macrophages as a therapy for liver diseases.[2] In subsequent years, numerous studies have provided new insights into the development, phenotypes, and functional roles of hepatic macrophages. This current review provides an update on the knowledge gained in recent years regarding the functions of hepatic macrophages in maintaining liver homeostasis, as well as the involvement of these cells in a multitude of processes associated with liver diseases, including exacerbation of injury, resolution of inflammation, tissue repair, pro- and antifibrogenesis, and pro- and antitumorigenesis. We also highlight novel perspectives on therapeutic strategies targeting hepatic macrophages to treat liver diseases.

HEPATIC MACROPHAGES IN LIVER HOMEOSTASIS

Subsets, origins and replenishment of hepatic macrophages in the steady state

KCs and MoMϕs are distinct subsets of macrophages in the liver that can be distinguished from each other based on their differential expression of cell surface markers. In mice, MoMϕs are $CD11b^{+}$, $F4/80^{intermediate\ (int)}$, $Ly6C^{+}$ and $CSF1R^{+}$, where KCs are $CD11b^{low}$, $F4/80^{high}$ and $Clec4F^{+}$.[1,3–6] MoMϕs are differentiated from circulating monocytes, which are derived from bone marrow (BM) $CX3CR1^{+}CD117^{+}Lin^{-}$ progenitor cells.[7] In mouse models of liver diseases, hepatic MoMϕs are divided into two main subpopulations according to Ly6C expression levels: $Ly6C^{high}$ and $Ly6C^{low}$ MoMϕs.

Resident KCs are the predominant hepatic macrophages in the healthy naïve liver. It is widely accepted that KCs originate from yolk sac-derived $CSF1R^{+}$ erythromyeloid progenitors (EMPs), which reside in the fetal liver during embryogenesis.[8] In mice, on embryonic days 10.5–12.5, yolk sac-derived EMPs develop into fetal liver monocytes, which give rise to KCs.[9,10] However, there is experimental evidence to support another scenario. On embryonic day 8.5, yolk sac EMPs develop into circulating macrophage precursors (pre-macrophages), which migrate to the nascent fetal liver in a CX3CR1-dependent manner before day 10.5 and subsequently give rise to KCs through regulation by an essential transcription factor, inhibitor of differentiation 3 (ID3).[8,11] KCs have a half-life of 12.4 days in mice and require replenishment for maintaining homeostasis.[12] KC replenishment in the steady state is independent of BM-derived progenitors but predominantly relies on self-renewal, which is tightly controlled by repressive transcription factors (MafB and cMaf).[8,13–15]

Recently, single-cell RNA-sequencing shed new light on the understanding of the heterogeneity of human hepatic macrophages. In the human liver, hepatic macrophages consist of $CD68^{+}MARCO^{+}$ KCs, $CD68^{+}MARCO^{-}$ macrophages, and $CD14^{+}$ monocytes.[16,17] $CD68^{+}MARCO^{+}$ KCs are characterized by enriched expression of genes involved in maintaining immune tolerance (e.g., VSIG4) and suppressing inflammation (e.g., CD163 and

[1]Department of Anesthesiology, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA and [2]Department of Hepatology and Gastroenterology, Charité University Medicine Berlin, Berlin, Germany
Correspondence: Cynthia Ju (changqing.ju@uth.tmc.edu) or Frank Tacke (frank.tacke@charite.de)
These authors contributed equally: Yankai Wen, Joeri Lambrecht

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HMOX1). $CD68^+MARCO^-$ macrophages have a similar transcriptional profile (e.g., C1QC, IL-18, S100A8/9) as recruited proinflammatory macrophages.[16] However, both $CD68^+MARCO^-$ macrophages and hepatic $CD14^+$ monocytes show significantly weaker proinflammatory responses than circulating $CD14^+$ monocytes.[17]

Functionality of hepatic macrophages during homeostasis

KCs play a critical role in maintaining homeostasis of the liver and the whole body through five major functions. These include (i) clearance of cellular debris and metabolic waste,[18–20] (ii) maintenance of iron homeostasis via phagocytosis of red blood cells (RBCs) and the subsequent recycling of iron,[21–24] (iii) regulation of cholesterol homeostasis through the production of cholesteryl ester transfer protein, which is important for decreasing circulating high-density lipoprotein-cholesterol levels and increasing very low-density lipoprotein-cholesterol levels,[25] (iv) mediation of antimicrobial defense,[26,27] and (v) promotion of immunological tolerance.[28,29]

Clearance of damaged or aged RBCs is crucial for systemic homeostasis. Studies in which $Na_2{}^{51}CrO_4$-labeled oxidized RBCs and $Na_2{}^{51}CrO_4$-labeled RBC-derived hemoglobin-containing vesicles were injected into mice have demonstrated that KCs are responsible for the rapid uptake of nearly half of the injected RBCs and RBC-derived vesicles. These phagocytic processes rely on the presence of polyinosinic acid- and phosphatidylserine-sensitive scavenger receptors on KCs.[20,21] An important outcome of the clearance of RBCs and their vesicles by KCs is the recycling of iron, which maintains iron homeostasis and prevents iron deficiency or iron toxicity. Moreover, KCs can directly take up iron and export it to hepatocytes for long-term storage through transferrin receptor and ferroportin, respectively.[24,30,31] In addition to RBC clearance, KCs also contribute to the clearance of aged platelets. Indeed, KC depletion by liposome-entrapped clodronate (CLDN) causes the accumulation of aged platelets in the blood, leading to impaired coagulation.[18] The expression of macrophage galactose lectin is important for KC-mediated platelet clearance.[18]

KCs reside along sinusoids and serve as the first-line defense against pathogens by efficiently recognizing and removing blood-borne Gram-positive bacteria. Complement receptor of immunoglobulin superfamily (CRIg), which is uniquely expressed on KCs in the liver, rapidly recognizes and binds to lipoteichoic acid (LTA), particularly on Gram-positive bacteria.[27]

KCs represent a major population of antigen presenting cells (APCs) in the liver. They are important for maintaining immunological tolerance of the liver through activating regulatory T cells (Tregs)[29] and suppressing effector T cell activation induced by other APCs.[28] In mice subjected to systemic delivery of low-dose particle-bound antigens, KC-associated antigen presentation induces $CD4^+$ T cell arrest and expansion of IL-10-expressing Tregs, leading to tolerogenic immunity and suppression of inflammation in the liver.[29] An in vitro coculture study demonstrated that KCs isolated from the livers of naïve mice can suppress dendritic cell (DC)-induced T cell activation by releasing prostaglandin E_2 (PGE_2) and 15d-PGJ_2.[28] Moreover, KCs pretreated with interferon gamma (IFNγ) acquire the ability to suppress T cell responses by expressing indoleamine 2,3-dioxygenase and Fas ligand in vitro.[32]

An interesting population of MoMϕs residing in the hepatic capsule at steady state has been described.[33] These liver capsular macrophages (LCMs) are $CD11b^+F4/80^+CD11c^+MHC\text{-}II^+CSF1R^+$ but negative for Ly6C, Clec4F and TIM4, suggesting that they are distinct from $Ly6C^+$ MoMϕs and KCs.[33,34] LCMs are replenished by circulating monocytes.[33] They recognize peritoneal bacteria accessing the liver capsule and promote the recruitment of neutrophils, thereby reducing hepatic pathogen loads. Depletion of LCMs by an anti-CSF1R antibody results in defective recruitment of neutrophils and increases hepatic dissemination of peritoneal pathogens,[33] suggesting that this specific population of capsular phagocytes protects pathogens from spreading across compartments.

HEPATIC MACROPHAGES IN LIVER DISEASES

Dynamic changes in macrophage subsets and their replenishment during liver diseases

A rapid loss of KCs occurs during liver injury upon infection with the DNA-encoding viruses vaccinia virus, murine cytomegalovirus[35] or the bacterium *Listeria monocytogenes*.[36] A reduction in the number of KCs is also observed in models of methionine/choline-deficient (MCD) diet-induced nonalcoholic steatohepatitis (NASH)[37] and hepatocellular carcinoma (HCC).[38] With regard to KC replenishment, it is believed that KCs may have the capacity to self-renew through proliferation,[39] probably due to colony stimulating factors.[5] This hypothesis, however, requires further investigation. MoMϕs are believed to be the major contributors to replenishment of the macrophage pool. Indeed, after selective depletion of Clec4F-expressing KCs, recruited MoMϕs differentiate into fully functional KCs, restoring the population of hepatic macrophages within 1 month.[6,40] As demonstrated by a novel mouse model of conditional KC depletion, monocytes can even acquire a "KC phenotype" within days. This process depends on the concerted actions of hepatic stellate cells (HSCs) and liver sinusoid endothelial cells (LSECs), which orchestrate monocyte engraftment and imprinting of the KC phenotype, including the transcription factors ID3 and liver X receptor-alpha (LXR-α).[41,42]

Depending on the signals expressed by the liver microenvironment, recruited MoMϕs can differentiate into cells of various phenotypes. For example, the recruitment of inflammatory $Ly6C^{high}$ MoMϕs specifically relies on the CCL2/CCR2, CCL1/CCR8, and CCL25/CCR9 signaling pathways, with the chemoattractants being secreted by activated KCs, HSCs, and LSECs.[43–47] Inhibition or elimination of these signaling pathways in mice leads to reduced MoMϕ recruitment, hepatic inflammation, and overall fibrosis.[43,48] However, it should be noted that MoMϕs are highly plastic, as highlighted by the potential of $Ly6C^{high}$ MoMϕs to switch toward a restorative $Ly6C^{low}$ phenotype.[49] Such a restorative phenotype can be induced by phagocytosis (e.g., of empty liposomes[49]) but also by exposure of MoMϕs to IL-4 and IL-33 derived from necrotic KCs.[36]

Aside from the proliferation of KCs and the recruitment and differentiation of MoMϕs, other cellular sources for hepatic macrophage replenishment have been suggested. Peritoneal macrophages, which are prenatally established and present in the peritoneal cavity, may be recruited through the visceral endothelium into liver tissue. In a model of sterile liver injury, mature peritoneal macrophages expressing CD102 and GATA6 have been found to migrate toward subcapsular liver tissue within 1 hour after injury. Furthermore, GATA6-deficient mice exhibit impaired macrophage recruitment and tissue regeneration.[50,51] Splenic macrophages have also been suggested to contribute to the hepatic macrophage pool upon liver injury. The spleen serves as a reservoir of monocytes that are capable of regulating immune responses during liver damage.[52] Indeed, the production and release of lipocalin-2 (LCN2)[53] and CCL2[54] by the spleen regulates monocyte infiltration into the liver, KC activation, and overall hepatic inflammation. Due to the limited knowledge concerning splenic macrophages, their involvement in liver diseases remains to be elucidated.

Last, due to the complex nature of liver diseases and the contribution of other organs to disease initiation and progression, extrahepatic macrophages may also play an important role. For example, lipid-associated macrophages (LAMs), which are $TREM2^+CD9^+CD68^+$ cells found around enlarged adipocytes during obesity, are important for preventing adipocyte hypertrophy and the loss of systemic lipid homeostasis in obesity.[55]

Interestingly, single-cell RNA-sequencing of liver tissues obtained from mice fed a high-fat diet (HFD) have revealed that $TREM2^+CD9^+CD68^+$ LAMs express gene signatures associated with lipid metabolism. These results suggest that macrophages in different tissues could respond similarly to a microenvironmental cue and thus may serve as therapeutic targets for metabolic disease.[55]

Of particular interest, in mice fed a NASH diet, embryonic KCs undergo cell death, allowing repopulation by monocyte-derived KCs.[56] The NASH diet induces significant changes in gene expression in KCs through LXR-mediated reprogramming, resulting in a partial loss of KC identity but increased expression of TREM2 and CD9.[56] Increased TREM2 expression is strongly associated with higher nonalcoholic fatty liver disease (NAFLD) activity scores, which reflect the severity of steatosis, inflammation, hepatocyte ballooning, and fibrosis.[57] Future studies are warranted to elucidate the underlying mechanisms by which TREM2 and $TREM2^+CD9^+$ KCs are involved in NASH development.

As almost all of the above studies relied on the use of mouse models, the relevance of these findings to humans requires further investigation. One of the debated topics concerning species differences is cell-type specific macrophage markers. Indeed, while murine KCs are mainly identified as $F4/80^+CD11b^{int}Clec4F^+$ cells,[58,59] single-cell RNA-sequencing data has defined the human KC population as $CD163^+MARCO^+CD5L^+TIMD4^+$.[60] Interestingly, a subpopulation of $TREM2^+$ macrophages was found in the fibrotic scars of human cirrhotic livers by single-cell RNA-sequencing and immunohistochemical studies.[61] A comprehensive analysis of early macrophage development during human embryogenesis suggested that yolk sac-derived primitive macrophages or embryonic liver monocytes as the major sources of tissue-resident macrophages in humans.[62]

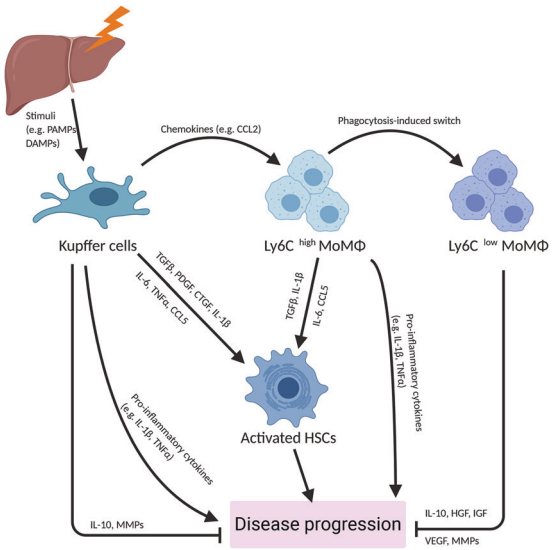

Fig. 1 The role of hepatic macrophages in liver diseases. Schematic overview of the roles of Kupffer cells and monocyte-derived macrophages in liver disease. CCL C-C chemokine ligand, CTGF connective tissue growth factor, DAMP damage-associated molecular pattern, HGF hepatocyte growth factor, HSC hepatic stellate cell, IGF insulin-like growth factor, IL interleukin, MMP matrix metallopeptidase, MoMϕ monocyte-derived macrophages, PAMP pathogen-associated molecular pattern, PDGF platelet-derived growth factor, TGF transforming growth factor, TNF tumor necrosis factor, VEGF vascular endothelial growth factor

The involvement of hepatic macrophages in the pathogenesis of liver diseases

Due to their central position in the hepatic microenvironment, their long cytoplasmic protrusions, and the high density of pattern recognition receptors (PRRs) on their surface, including Toll-like receptors (TLRs) and nucleotide binding oligomerization domain-like receptors (NLRs), KCs act as first-line responders upon liver injury (Fig. 1).[59] Indeed, a plethora of signals associated with the initiation and progression of liver disease may lead to the activation of KCs, such as the following: (i) The release of reactive oxygen species (ROS) and damage-associated molecular patterns (DAMPs), e.g., high mobility group box 1 (HMGB1), mitochondrial DNA (mtDNA), and ATP, by damaged hepatocytes undergoing apoptosis or necrosis. (ii) Pathogen-associated molecular patterns (PAMPs), which are the result of increased intestinal permeability as well as changes in the gut microbiome and reach KCs in the liver sinusoids via the portal vein. Examples of relevant PAMPs include lipopolysaccharide (LPS), LTA, and β-glucan.[63] (iii) Enhanced expression of hypoxia-inducible factor (HIF)-1α (or similar transcription factors related to environmental stress) caused by a hypoxic liver environment, which is associated with progressive liver diseases.[64] (iv) Metabolic changes in hepatocytes, caused by excessive uptake of dietary fats and carbohydrates, which are associated with high hepatic levels of triglycerides, cholesterol[65] and various metabolites such as succinate,[66] which is known to promote TLR signaling and inflammasome activation. (v) Extracellular vesicles, which are derived from various cells of the liver environment and contain proinflammatory stimuli such as mitochondrial double-stranded RNA,[67] microRNA (miRNA)-27,[68] and heat shock protein 90 (HSP90).[69]

Release of cytokines and chemokines. As the initial sensors of liver injury, KCs secrete a variety of chemokines to recruit monocytes and other leukocytes.[70] KCs are a major source of CCL2,[71,72] which recruits $CCR2^+$ monocytes into the diseased liver (Fig. 1). KCs also secrete CXCL1, CXCL2, and CXCL8 to attract neutrophils,[71] which contribute to hepatic ischemia/reperfusion (I/R) injury and heat-induced liver injury.[70,73,74] Similarly, infiltrated $Ly6C^{high}$ MoMϕs also release chemokines and contribute to leukocyte recruitment during liver diseases. For example, in mouse models of liver fibrosis induced by carbon tetrachloride (CCl_4) and an MCD diet, $Ly6C^{high}$ MoMϕs express CXCL16 and promote the recruitment of $CXCR6^+$ natural killer T (NKT) cells, which exacerbate inflammation and fibrogenesis.[75] In mice fed a HFD, $Ly6C^{high}$ MoMϕs produce CCL5 and CXCL9 in a S100 calcium-binding protein A9 (S100A9)-dependent manner. These chemokines lead to the hepatic recruitment of both $CD4^+$ and $CD8^+$ T cells, which contribute to insulin resistance.[76,77] Studies of the chronically inflamed livers of patients with alcoholic liver disease (ALD), NASH, primary biliary cholangitis or primary sclerosing cholangitis have also shown that intermediate $CD14^{high}CD16^+$ monocytes (close to $Ly6C^{high}$ MoMϕs in the murine liver), which are derived from infiltrated classic $CD14^{high}CD16^-$ monocytes, secrete proinflammatory cytokines and chemokines, such as TNFα, IL-1β, CCL1 and CCL2.[78]

The contribution of KCs and $Ly6C^{high}$ MoMϕs to liver inflammation and injury observed in a study often depends on the disease and model used. For example, in a mouse model of steatohepatitis induced by a combination of a HFD and alcohol, inflammatory MoMϕs, but not KCs, are activated to produce proinflammatory TNFα and IL-1β in a Notch1-dependent manner.[79] In a mouse model of acetaminophen (APAP)-induced liver injury (AILI), it appears that $Ly6C^{high}$ MoMϕs exhibit a stronger proinflammatory phenotype than KCs. Compared with KCs, $Ly6C^{high}$ MoMϕs express higher levels of complement factors (C3), proinflammatory cytokines (IL-1β, IL-18 and MIF) and markers of a proinflammatory phenotype (CYBB, TREM1/2 and S100A8/A9).[76]

The TLR4 and TLR9 signaling pathways are important for mediation of the production of proinflammatory cytokines by hepatic macrophages.[80–83] TLR4/MyD88 signaling is responsible

for sensing DAMPs (e.g., HMGB1 and mtDNA from damaged cells), saturated fatty acids (e.g., palmitate) and gut-derived endotoxin. TLR4 deficiency reduces proinflammatory cytokines and liver injury in mouse models of AILI and ALD.[81,84] Another study demonstrated that hepatic inflammation and injury are attenuated in endotoxin-resistant TLR4 mutant mice with diet-induced NASH.[80] Similarly, as a pattern recognition receptor, TLR9 recognizes PAMPs and DAMPs (e.g., mtDNA). It has been demonstrated that TLR9 and stimulator of interferon genes (STING) synergistically trigger a proinflammatory response to mtDNA in macrophages during NASH development.[85] In contrast, TLR9 deletion or pharmacological antagonism results in an attenuated response to bacterial DNA and mtDNA, leading to reduced IL-1β production, steatosis and liver injury in models of diet-induced NASH.[82,83]

Inflammasome activation. The inflammasome is a multiprotein complex that can sense danger signals from pathogens and damaged cells via TLRs and NLRs. Inflammasome activation triggers caspase-1-mediated cleavage and maturation of the cytokines IL-1β and IL-18.[86] In the liver, gut-derived PAMPs, cell damage-induced DAMPs (e.g., ATP), crystals (e.g., cholesterol), palmitic acid, and ROS are well-characterized signals that trigger inflammasome activation in macrophages.[87–90]

Activation of inflammasomes, including the NLR family pyrin domain containing 3 (NLPR3) inflammasome and absent in melanoma 2 (AIM2) inflammasome, in macrophages amplifies tissue inflammation and hepatocyte damage, thereby contributing to various liver diseases.[91] In mouse models of hepatic I/R injury, inflammasomes are activated by ROS, HMGB1 (via TLR4) and histones (via TLR9) in KCs and promote liver injury.[92–94] In other models of acute liver injury induced by D-galactosamine (GalN)/LPS, CCl_4 or LPS, inflammasomes are activated in macrophages through an increase in mitochondrial ROS levels resulting from autophagy deficiency.[95–97] However, studies on the role of inflammasome activation in AILI have yielded controversial results. It has been reported that extracellular ATP activates (via P2X7) NLRP3 in KCs and triggers IL-1β release, exacerbating liver injury.[98,99] In support of this finding, P2X7-deficient mice exhibit decreased liver necrosis after APAP challenge.[98] However, another study demonstrated that genetic deletion of NLPR3 or caspase-1 or antibody-mediated neutralization of IL-1β does not affect APAP-induced inflammation and injury in the liver.[84]

The role of macrophage inflammasome activation in chronic liver diseases, such as ALD and NASH, has also been reported.[100] IL-1β, which is released as a result of inflammasome activation in KCs, plays a critical role in mediating alcohol-induced steatosis, inflammation, and liver injury.[101] Deletion of caspase-1 or caspase-1 adaptor (ASC) in mice leads to impaired IL-1β production, thereby ameliorating ALD.[101] With regard to NASH, in vitro studies have shown that the NLRP3 inflammasome can sense lipotoxicity-associated increases in intracellular ceramide levels, cholesterol crystals, saturated fatty acid content, mtDNA levels and ROS content, causing the induction of caspase-1 in macrophages and subsequently contributing to IL-1β production.[90,102–105] In mouse models of NASH induced by a variety of diets, including an atherogenic diet, MCD, HFD and Western diet, NLRP3 inflammasome activation and subsequent IL-1β production exacerbate inflammatory responses while increasing the levels of IL-6 and CCL2 and enhance the numbers of infiltrated MoMϕs and neutrophils.[90,105] Furthermore, genetic deletion or pharmacological inhibition of NLRP3 in mice significantly suppresses tissue inflammation and attenuates the pathological features of NASH, such as fibrosis and insulin resistance.[90,102]

The role of inflammasome activation in KCs during pathogen infection depends on the specific bacterial or viral stimulus. Pathogens such as *Francisella tularensis* and *Salmonella typhimurium* cause inflammasome activation in KCs and exacerbate liver injury.[106–108] However, inflammasome activation by *Listeria monocytogenes* seems to promote the killing of bacteria. P2X5 deficiency attenuates *Listeria*-induced inflammasome activation and the bacterial-killing capacity of hepatic macrophages, which can be restored by IL-1β or IL-18.[109] Different types of viruses also have varying effects on inflammasome activation in macrophages. The HBeAg protein of hepatitis B virus (HBV) represses NLRP3 and pro-IL-1β expression in macrophages via inhibition of NF-κB activation.[89,108] Moreover, through attenuating ROS production, HBeAg suppresses caspase-1 activation and IL-1β maturation, resulting in suppression of the antiviral immune response.[89,108] In contrast, the core protein of hepatitis C virus (HCV) triggers inflammasome activation and IL-1β production by macrophages through the induction of potassium and calcium mobilization, driving proinflammatory responses to HCV.[110,111] In a model of murine hepatitis virus strain-3 (MHV-3)-induced fulminant hepatitis, macrophage NLRP3 inflammasome activation by ROS released from MHV-3-infected macrophages aggravates hepatitis.[112]

Inflammasome activation is observed in cholestatic liver diseases.[113] Animal studies have demonstrated that macrophage inflammasome activation exacerbates bile duct ligation (BDL)-induced cholestatic liver injury.[113,114] KC depletion by CLDN or treatment with an NLRP3 inhibitor significantly attenuates α-naphtylisocyanate (ANIT)-induced cholestatic liver injury, further supporting a role for macrophage inflammasome activation in the pathogenesis of the disease.[115] Studies investigating whether bile acids play an important role in triggering macrophage inflammasome activation have yielded controversial findings. The hydrophobic bile acids chenodeoxycholic acid and deoxycholic acid have been found to induce macrophage NLRP3 inflammasome activation.[114,116] In contrast, major endogenous bile acids, such as taurocholic acid, do not directly activate inflammasomes in macrophages or hepatocytes.[113] Moreover, bile acid-induced macrophage inflammasome activation appears to be independent of the bile acid receptor TGR5, as TGR5 actually causes NLRP3 ubiquitination and inhibits inflammasomes.[117]

Crosstalk with other cells. The contribution of hepatic macrophages to the pathogenesis of various liver diseases is manifested by their interaction with other cell types in the liver, including HSCs, LSECs, neutrophils, and platelets. For example, HSCs are the main effector cells that cause hepatic fibrosis; nonetheless, hepatic macrophages modulate HSC viability and activation through releasing cytokines and other soluble factors, thereby playing an important role in both fibrogenesis and the resolution of fibrosis.[118,119] Macrophage-HSC crosstalk will be discussed in more detail later in the "*Fibrosis*" section.

Macrophage-LSEC interactions play an important role in angiogenesis. In mice with chronic liver injury induced by BDL or CCl_4, inflammatory MoMϕs accumulate in the injured liver, colocalizing with newly formed blood vessels in portal vein tracts and promoting vessel sprouting through the release of vascular endothelial growth factor A (VEGF-A) and matrix metallopeptidase 9 (MMP9).[120] In partial hepatectomy (PHx)-induced liver regeneration, KCs release TNFα, which activates LSECs to express intracellular adhesion molecule 1 (ICAM-1). ICAM-1-expressing LSECs facilitate the adhesion and transmigration of monocytes, which promote vascular growth and support liver regeneration. Recruited monocytes serve as chaperones for endothelial sprouting by locally secreting proliferative factors (Wnt5a and Ang-1) and activating Notch1 to stabilize stalk cells.[121]

The macrophage-neutrophil interaction contributes to neutrophil recruitment and switching of the macrophage phenotype. Upon liver injury, neutrophils are recruited by CXCL1, CXCL2, and CXCL8 derived from KCs.[71,122] During acute liver injury, infiltrated neutrophils can contribute to hepatic inflammation and aggravate liver diseases by producing ROS, secreting proinflammatory

cytokines such as IL-1β and TNFα and recruiting inflammatory monocytes.[70,123] More recently, the importance of neutrophils in supporting macrophage-dependent repair mechanisms was reported.[124] In infectious diseases, infiltrated neutrophils play a crucial role in bacterial killing through the release of antimicrobial granule proteins and/or the formation of neutrophil extracellular traps (NETs).[70,125] A recent study also revealed a function for neutrophils in facilitating the differentiation of Ly6C^{high} MoMϕs to Ly6C^{low} MoMϕs.[126] Details are discussed in the "*Phenotype switching*" section.

Platelets contribute to inflammation and injury during acute liver injury and are also involved in hepatoprotective and hepatotoxic processes during chronic liver diseases.[127] Studies of macrophage-platelet interactions have begun to emerge. In NASH, the interaction of platelets with KCs through platelet glycoprotein Ib alpha chain (GPIbα), rather than platelet aggregation, contributes to tissue inflammation and disease progression.[128] Blocking this interaction with an anti-GPIbα antibody alleviates tissue inflammation, injury, steatosis, and fibrosis during NASH development.[128] In hepatic I/R injury, the KC-platelet interaction contributes to exacerbation of liver injury, especially in steatotic livers.[129,130] Moreover, the KC-platelet interaction is critical for first-line defense against bacterial infection. In mice infected by bacteria, such as *Bacillus cereus* and methicillin-resistant *Staphylococcus aureus* (MRSA), it has been observed that platelets switch from a transient "touch-and-go" interaction with KCs to sustained GPIIb/IIIa-mediated adhesion to KCs via von Willebrand factor (VWF). The two cell types collaborate to eradicate infectious bacteria.[131] Although platelet recruitment is important for limiting bacterial infection, prolonged accumulation of platelets increases the risk of aberrant and damaging thrombosis throughout the liver.[132] During *Salmonella Typhimurium* infection, inflammation in the liver triggers thrombosis within blood vessels via ligation of C-type lectin domain family 1 member B (CLEC-2) on platelets by podoplanin expressed by hepatic macrophages, including MoMϕs and KCs.[133] Thus, when targeting macrophage-platelet interactions, both the immunological consequences of these cellular interactions and their related effects on blood flow and thrombogenesis need to be considered.

Roles of macrophages in resolving inflammation during liver injury

Macrophage death. Hepatic macrophages undergoing cell death, such as pyroptosis and necroptosis, are often observed during pathogen infection and sterile liver injury, and death of hepatic macrophages represents an important mechanism of bacterial clearance and inflammation resolution.[134] During infection by flagellin-expressing *Salmonella typhimurium*, *Legionella pneumophila* or *Bukholderia thailandensis*, caspase-1-induced pyroptosis of macrophages causes the release of ROS, which subsequently promotes the bacteria-killing activity of neutrophils.[135] Both *Listeria monocytogenes* and *Salmonella enterica* induce early rapid necroptosis of KCs in vivo. Necroptotic KCs release IL-1β,, which induces IL-33 production by hepatocytes. IL-33, together with basophil-derived IL-4, promotes alternative activation of anti-inflammatory MoMϕs, which replenish KCs and restore liver homeostasis.[36] These findings indicate the crucial role of macrophage death in orchestrating the inflammatory responses and tissue repair processes during bacterial infection of the liver.

The role of macrophage death in resolving inflammation in sterile liver diseases remains controversial. In mice with alcoholic liver injury, after 3 days of feeding with an ethanol-containing liquid diet, KCs undergo forkhead box O3 (FOXO3)-dependent apoptosis, which promotes Ly6C^{high} MoMϕs to differentiate into restorative Ly6C^{low} MoMϕs. Failure of KCs to undergo apoptosis in the absence of FOXO3 leads to hyperinflammation and increased sensitivity to liver injury induced by ethanol feeding plus LPS treatment.[136] In contrast, two independent studies demonstrated the deleterious impact of macrophage death on hepatic I/R injury. One study reported a rapid loss of KCs through receptor-interacting protein kinase 1 (RIP1)-dependent necroptosis. RIP1 inhibition by necrostatin-1s protects KCs from I/R-induced depletion, resulting in suppression of inflammation and protection of the liver from I/R injury.[137] Another study observed gasdermin D (GSDMD)-dependent pyroptosis of KCs after I/R injury. Similarly, mice with GSDMD deletion in myeloid cells exhibit attenuation of inflammation and alleviation of I/R injury.[138]

Phenotype switching. Infiltrated proinflammatory CCR2^{+}Ly6C^{high} MoMϕs usually represent the predominate population of macrophages in the early phases of liver injury.[45] Differentiation of these Ly6C^{high} MoMΦs into restorative Ly6C^{low} MoMϕs indicates the transition of an inflammation/injury phase to a resolution/repair phase.[49,126,139,140] Ly6C^{low} MoMϕs show an anti-inflammatory and restorative phenotype by expressing MMPs (MMP9, MMP12, and MMP13), growth factors (HGF and IGF), and phagocytosis-related genes (MARCO) (Fig. 1).[49,76,141] Expression of these genes enables wound healing, clearance of dead cells, and promotion of hepatocyte proliferation, thereby allowing the liver to return to homeostasis after injury and fibrosis.[49,139,142,143] In mice challenged by APAP overdose, preventing monocyte infiltration by neutralization of CCR2 results in the absence of Ly6C^{low} MoMϕs and thus a lack of tissue inflammation resolution and the accumulation of late apoptotic neutrophils.[143] Conversely, injection of BM-derived alternative activated macrophages, which are primarily Ly6C^{low} MoMϕs, stimulates hepatocyte proliferation and accelerates recovery of the liver from APAP-induced necrosis.[142]

Phagocytosis of dead cells (efferocytosis) is a major mechanism that promotes the switch of Ly6C^{high} MoMϕs to Ly6C^{low} MoMϕs (Fig. 1). In vitro coculture experiments have demonstrated that phagocytosis of apoptotic hepatocytes by Ly6C^{high} MoMϕs induces their switch to Ly6C^{low} MoMϕs.[141] The efferocytosis-driven phenotype switch of Ly6C^{high} MoMϕs is mediated by the STAT3/IL-10/IL-6 signaling pathway.[144] A recent study demonstrated that IL-4 and/or IL-13 in conjunction with c-met proto-oncogene tyrosine kinase (MerTK)- and/or AXL receptor tyrosine kinase (AXL)-dependent efferocytosis are necessary to drive the differentiation of MoMϕs into an anti-inflammatory and tissue reparative phenotype.[145] This finding is further supported by two studies of liver injury. One study demonstrated that during bacterial infection, basophil-derived IL-4 promotes the switch of Ly6C^{high} MoMϕs to anti-inflammatory MoMϕs, thereby resolving inflammation and restoring tissue homeostasis.[36] Another study demonstrated that mice deficient in MerTK exhibit a reduced number of Ly6C^{low} MoMϕs, correlating with increased accumulation of late neutrophils and impaired inflammation resolution upon APAP challenge.[146]

There is experimental evidence that aside from efferocytosis, interactions with other cell types underlie macrophage phenotype switching. For example, upon inflammatory stimulation by LPS, Ly6C^{high} MoMϕs activate neutrophils to produce ROS,[143] which in turn mediates the switch of Ly6C^{high} MoMϕs to Ly6C^{low} MoMϕs via Ca^{2+}-CaMKKβ-dependent AMPK activation.[126] This switch is prevented by the depletion of neutrophils with an anti-Ly6G antibody or by genetic deletion of NADPH oxidase 2 (Nox2), which is required for the production of ROS by neutrophils.[126,140]

In mice infected with *Schistosoma mansoni*, the switch of Ly6C^{high} MoMϕs to Ly6C^{low} MoMϕs appears to be facilitated by CD4^{+} T cells, as depletion of CD4^{+} T cells blocks this phenotypic switch.[147] T cell-derived IL-4 may be an important mediator of macrophage differentiation. It has been shown that in the presence of IL-4, Ly6C^{low}F4/80^{int} MoMϕs further differentiate into F4/80^{high} macrophages. Failure of the conversion of Ly6C^{low}F4/80^{int} MoMϕs to F4/80^{high} macrophages leads to dysregulation of inflammation, disruption of liver granuloma architecture and increased mortality.[148]

Release of proresolving mediators. During the resolution phase, anti-inflammatory and prosurvival cytokines are released from macrophages. IL-10 produced by macrophages has been reported to protect against liver inflammation and injury in both acute and chronic liver diseases. KC depletion by CLDN exacerbates liver injury after APAP challenge and I/R treatment through a reduction in IL-10 expression.[149,150] Liver injury is exacerbated in IL-10-deficient mice after APAP challenge, as in KC-depleted mice.[149] In contrast, exogenous IL-10 can alleviate KC depletion-induced exacerbation of liver inflammation and damage caused by I/R.[150] In liver fibrosis, activated HSCs induce MoMϕs to produce IL-10, which in turn suppresses HSC activation and the expression of αSMA and Col1a1.[151]

IL-6, which signals through STAT3, is an important hepatoprotective cytokine that can promote hepatocyte survival and proliferation, inhibit steatosis, and prevent insulin resistance.[152] Hepatic macrophages are major sources of IL-6. In AILI, depletion of KCs abrogates IL-6 production, thereby exacerbating liver injury.[149] A recent study demonstrated that HIF-2α in hepatic macrophages induces IL-6 production and promotes hepatocyte survival through STAT3 activation during AILI.[153] In ALD, IL-6 produced by KCs triggers hepatocyte senescence, rendering hepatocytes resistant to alcohol-induced apoptosis as a protective mechanism.[154]

To promote tissue repair, macrophages secrete mediators involved in tissue growth (e.g., IGF) and remodeling (e.g., MMPs) in liver diseases, including AILI, ALD, and fibrosis.[49,141,155] For example, in a mouse model of fibrosis induced by thioacetamide (TAA) or CCl_4, macrophages are the major sources of MMP9 and MMP13, which promote the resolution of fibrosis. Studies of CLDN treatment in wild-type mice or diphtheria toxin (DT)-induced macrophage depletion in CD11b-DT receptor transgenic mice during fibrosis resolution demonstrate that a loss of macrophages results in delayed resolution of fibrosis and reduced hepatic expression of MMP9 and MMP13.[156,157] Moreover, adoptive transfer of wild-type KCs relieves fibrolysis in MMP9-deficient mice.[156]

Role of macrophages in physiological and pathological repair

Liver repair and regeneration. Macrophages are the most extensively studied immune cells in the context of liver regeneration. Both KCs and MoMϕs play important roles in promoting hepatocyte proliferation. KC depletion by CLDN impairs DNA synthesis in the proliferating hepatocytes of mice during liver repair after BDL- or alcohol-induced injury.[158,159] The beneficial role of KCs in liver regeneration was further confirmed in a mouse model of noninjury-induced PHx. KC depletion by CLDN attenuates hepatocyte proliferation and delays liver regeneration after PHx.[160–164] KC-depleted mice exhibit reduced production of TNFα and IL-6 after PHx,[161–163] and both cytokines are important for the initiation of hepatocyte proliferation.[165–167] Moreover, TNFα enhances KC production of IL-6,[166,168] which directly induces hepatocyte entry into the cell cycle via STAT3 activation.[165,167]

During severe liver injury involving significant loss of hepatocytes, hepatic progenitor cells (HPCs, also termed oval cells) play an important role in hepatocellular regeneration.[169] KC depletion by CLDN impairs HPC-mediated differentiation into hepatocytes in two rodent models of liver regeneration: one triggered by 2-acetylaminofluorene (2-AAF) treatment plus PHx and the other caused by a choline-deficient, ethionine-supplemented (CDE) diet. The underlying mechanism involves reduced expression of TNFα, IL-6 and TNF superfamily member 12 (TWEAK) due to KC depletion.[170–172] In mice treated with a CDE diet, macrophages produce Wnt3a as a result of the clearance of hepatocyte debris, and Wnt3a is an important driving force of HPC differentiation toward hepatocytes.[173]

Aside from KCs, $CCR2^+$ MoMϕs have also been reported to promote liver regeneration. In mice, inhibition of MoMϕs recruitment resulting from impairment of myeloid CCR2 signaling results in attenuation of hepatocyte proliferation in CCl_4-induced hepatotoxicity.[174] Conditioned medium from $Ly6C^{low}$ MoMϕs, but not $Ly6C^{high}$ MoMϕs, can induce hepatocyte proliferation in vitro.[126] Furthermore, after PHx, the number of MoMϕs is increased in the liver, while the number of KCs remains unchanged.[175,176] When hepatic infiltration of MoMϕs is impaired by BM irradiation, a CCR2 antagonist or CCR2 deletion, hepatocyte proliferation is attenuated after PHx.[175,177] The underlying mechanism of MoMϕ-induced hepatocyte proliferation involves IL-6, as transferring wild-type BM to IL-6-deficient mice restores normal hepatocyte proliferation after PHx.[178]

The role of MoMϕs in HPC-induced liver regeneration has been reported as well. Adoptive transfer of BM-derived monocytes restores HPC differentiation into hepatocytes in KC-depleted mice fed a CDE diet.[171] Even in the healthy liver, adoptive transfer of BM-derived monocytes causes the expansion and differentiation of HPCs into functional parenchyma by activating TWEAK.[179]

Fibrosis. Liver fibrosis is a common pathological feature of most chronic liver diseases. Persistent or repetitive liver injury is often accompanied by dysregulated wound healing and tissue repair, resulting in excessive deposition of extracellular matrix (ECM) or failure of inflammatory resolution in the ECM. Hepatic macrophage depletion alleviates fibrogenesis in mice,[44,180–183] indicating a profibrogenic role of KCs (Fig. 1). First, in the early stage of liver injury, KCs secrete CCL2 to recruit proinflammatory and profibrogenic MoMϕs.[44,45,184,185] Second, KCs can directly activate HSCs via growth factors (TGFβ, PDGF and CTGF).[186–188] Third, KCs release proinflammatory cytokines and chemokines (TNFα, IL-1β, IL-6 and CCL5) to interact with HSCs to establish a profibrogenic niche.[185] HCV-exposed KCs secrete TNFα, inducing NLRP3 inflammasome activation in HSCs and subsequent production of IL-1β by these cells,[189] which drives the progression of NASH and alcoholic steatohepatitis (ASH).[90,101] An in vitro study of cocultured BDL-treated HSCs and hepatic macrophages isolated from mice revealed that macrophage-derived IL-1β and TNFα directly promote NK-κB activation in HSCs and support their survival. In mice with BDL-induced liver fibrosis, KC depletion decreases IL-1β and TNFα production and increases HSC death, thereby attenuating fibrogenesis.[190] In addition, an in vitro study demonstrated that HCV-exposed KCs produce CCL5, which induces fibrogenic activation of $CCR5^+$ HSCs through ERK phosphorylation.[189] KCs are also able to produce IL-6, which stimulates HSC proliferation, as well as the profibrotic tissue inhibitor of metalloproteinases 1 (TIMP1) through a p-38 phosphorylation-dependent mechanism.[191,192]

The pro- and antifibrogenic functions of MoMϕs reflect their differentiation stages (Fig. 1). Using CD11b-DTR transgenic mice, studies have shown that deletion of infiltrating MoMϕs during fibrogenesis results in reduced HSC activation and ECM deposition, whereas deletion of MoMϕs during regression of fibrosis impairs ECM degradation, thereby exacerbating fibrosis.[49,193] At early time points after their recruitment into the injured liver, $Ly6C^{high}$ MoMϕs exhibit a proinflammatory (TNFα, IL-1β, IL-6, CCL2 and CCL5) and profibrogenic (IL-13) phenotype and may directly activate HSCs in a TGFβ-dependent manner.[45,49,78,190] Unlike $Ly6C^{high}$ MoMϕs, $Ly6C^{low}$ MoMϕs appear to play an antifibrotic role. In experimental models of liver fibrosis induced by repetitive CCl_4 treatment or an MCD diet, administration of a CCL2 inhibitor prevents the influx of $Ly6C^{high}$ MoMϕs and causes an increase in the proportion of $Ly6C^{low}$ MoMϕs. As a result, fibrogenesis is attenuated, leading to increased resolution of fibrosis.[194] In another study of CCl_4-induced reversible fibrosis, activation of macrophage phagocytosis by the injection of liposomes was shown to induce the switch of $Ly6C^{high}$ MoMϕs to $Ly6C^{low}$ MoMϕs, resulting in accelerated regression of liver fibrosis.[49] Together,

these studies unveil a critical role for Ly6C^{low} MoMϕs in promoting the resolution of fibrosis during chronic liver injury.

Recently, LC3-associated phagocytosis (LAP), a noncanonical form of autophagy that triggers the switch of MoMϕs to an anti-inflammatory phenotype, was reported in patients with cirrhosis.[195] Studies involving pharmacological inhibition of LAP in monocytes isolated from patients with cirrhosis or genetic disruption of LAP in mice treated with CCl_4 have demonstrated that LAP attenuates inflammation through FcγRIIA-mediated activation of the anti-inflammatory Src homology region 2 domain-containing phosphatase-1 (SHP1)/inhibitory immunoreceptor tyrosine-based activation motif (ITAMi) pathway.[195] In contrast, mice overexpressing human FcγRIIA in myeloid cells show increased LAP activation, resulting in resistance to inflammation and CCl_4-induced liver fibrosis. Moreover, activation of LAP is abolished in monocytes from patients with acute-on-chronic liver failure and can be restored by specifically targeting ITAMi signaling with anti-FcγRIIA $F(ab')_2$ fragments or by intravenous injection of immunoglobulin (IVIg).[195] Together, these studies suggest a possible approach for targeting macrophages to induce anti-inflammatory and antifibrotic effects.

Cancer. Approximately 75–85% of all cases of primary liver cancer are HCC, which is the fourth leading cause of global cancer-related death.[196] The accumulation of hepatic macrophages in HCC-affected liver tissue of mouse and human origin and its correlation with HCC progression and poor prognosis[197,198] suggest the importance of macrophages in this pathology. Indeed, tumor-associated macrophages (TAMs) seem to have an inherent dominant protumorigenic character.[199] Moreover, through secretion of a plethora of proangiogenic factors (VEGF, PDGF, and TGFβ) and cell proliferation stimuli (IL-1β, IL-6, CCL2, TNF, and VEGF), TAMs strongly favor tumor growth and development.[2] In addition, acceleration of the migratory potential of HCC cells is induced by TAM-derived $\alpha_M\beta_2$ (CD11/18)-containing exosomes, which have been reported to activate the MMP9 signaling pathway.[200] Through the production of various cytokines, including CCL17, CCL18, and CCL22, TAMs attract Tregs to the tumor environment, thereby hampering cytotoxic T cell activation and thus promoting tumor development.[201]

Mouse models of hepatocarcinogenesis and observations of human tissues have indicated that macrophage subsets in the liver have stage-dependent effects. While KCs are the major phagocyte population in the (noncirrhotic) tumor environment during early HCC, a shift is observed toward liver infiltrating macrophages once the primary tumor is established. KCs can promote early tumor activity through different mechanisms: First, enhanced expression of PD-L1[202] and galectin-9[203] allows interactions with PD-1 and TIM3, respectively, resulting in repression of immunogenic T cell activation. Second, HCC signaling causes the upregulation of TREM1 on KCs, leading to the recruitment of CCR6$^+$Foxp3$^+$ Tregs[204] and thus suppressing the cytotoxic T cell response. Finally, KCs recruit platelets through hyaluron-CD44 binding, an essential step in hepatocarcinogenesis. Inhibition of the cargo-carrying ability, adhesion, and activation of platelets results in reduced KC activation and carcinogenesis in mouse models of HCC.[128] The later stages of HCC development are primarily promoted by MoMϕ-mediated suppression of NK cell function.[205]

Although liver macrophages are mainly believed to stimulate HCC development, some studies suggest they also have the potential to inhibit tumor growth. Indeed, hepatic macrophages have been reported to stimulate CD4$^+$ T cells to destroy precancerous senescent hepatocytes, thereby preventing tumor development.[206] The importance of CD4$^+$ T lymphocytes was further shown in NAFLD models, in which dysregulated lipid metabolism causes the selective loss of CD4$^+$ but not CD8$^+$ T lymphocytes, eventually leading to acceleration of hepatocarcinogenesis.[207] However, due to contradictory results between distinct studies, these antitumorigenic effects demand further elucidation, especially considering the heterogeneity of the TAM population.

THERAPEUTIC APPROACHES FOR TARGETING MACROPHAGES IN LIVER DISEASES

In the search for novel therapies for liver disease, multiple approaches that target distinct key pathways involved in disease initiation and progression are being explored.[208] Due to the major implication of liver macrophages in normal tissue homeostasis, their role as first-line responders upon liver damage, and their dual promoting and inhibitory functions in liver disease, hepatic macrophages are intriguing therapeutic targets. While most macrophage-based therapies have only been tested in experimental animal models, some have already been evaluated in clinical trials. Approaches with targeting the inflammatory system can often be categorized as into those that (i) hamper inflammatory cell (monocyte and macrophage) recruitment, (ii) inhibit macrophage activation, and (iii) shape macrophage function and polarization[2,209] (Fig. 2). More recently, cell-based therapies involving autologous macrophage infusions have been tested in patients with compensated liver cirrhosis.[210] The prospect of utilizing macrophages as agents for cell-based therapies has been reviewed elsewhere.[211]

Inhibition of inflammatory cell recruitment

As previously mentioned, the recruitment of proinflammatory MoMϕs to the injured liver relies on the chemoattractant properties of several chemokines secreted by activated liver cells; CCL2/CCR2,[44] CCL5/CCR5[212], and CCL1/CCR8[43] are examples of several important chemoattractive axes. Interference with chemokine signaling could thus represent an interesting therapeutic approach that has proven efficacious in various experimental rodent models[48,185,213] and can be achieved through different means, including monoclonal antibodies, receptor antagonists, aptamer molecules, and small-molecule inhibitors.[209] This latter approach, especially intervention with chemokine signaling pathways by inhibitory drugs, has been extensively studied. In particular, cenicriviroc (CVC), a dual CCR2/CCR5 inhibitor, has been shown to efficiently block CCL2-mediated monocyte recruitment and to exert anti-inflammatory and antifibrotic effects in various mouse models.[185,213,214] These results encouraged its

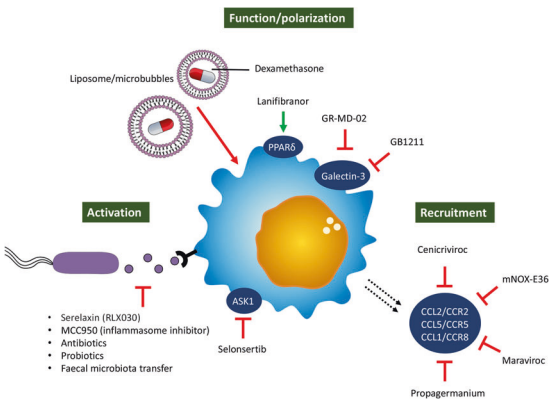

Fig. 2 Macrophage-based therapeutic approaches for liver disease. Schematic overview of the different therapeutic approaches that focus on the inflammatory system divided into those that hamper inflammatory cell recruitment, those that inhibit macrophage action, and those that shape macrophage function and polarization. ASK-1 apoptosis signal-regulating kinase 1, PPAR peroxisome proliferator-activated receptor, CCL C-C chemokine ligand, CCR C-C chemokine receptor

advancement to clinical trials evaluating the efficacy in NASH patients with liver fibrosis. After 1 year of CVC treatment, a significant number of NASH patients responded well to the treatment, showing a statistically significant improvement in the histological stage of fibrosis,[215] and these positive effects were maintained in responders in the 2nd year of treatment.[216] Currently, a phase 3 trial of CVC including ~2000 patients is ongoing (NCT03028740). Other inhibitors of chemoattractant axes include propagermanium, a CCR2 inhibitor,[217] mNOX-E36, an RNA-aptamer molecule that inhibits CCL2,[194] and maraviroc, a CCL5/RANTES inhibitor,[218] which all provoke disease amelioration in murine NAFLD/NASH models.

Recently, G protein-coupled receptor 84 (GPR84), a receptor for medium-chain fatty acids, was found to be upregulated on myeloid immune cells under inflammatory conditions. Enhanced GPR84 expression has been suggested to promote the inflammatory activation and phagocytic capacity of both human and murine macrophages.[219] Inhibition of GPR84 by small-molecule antagonists in mouse models of acute and chronic liver injury hampers the recruitment of inflammatory cells to the site of injury and an overall reduction in hepatic inflammation and fibrosis.[220]

Inhibition of macrophage activation

Significant changes in the microbial gut composition and increased intestinal permeability, both of which are characteristics of progressive liver disease, cause increases in the levels of endotoxins (e.g., LPS) that reach the liver via portal blood flow. These PAMPs, together with DAMPs derived from damaged liver cells, activate liver-resident macrophages through PRRs, among which the importance of TLR4 is well documented. Indeed, genetic depletion of TLR4 has a preventive effect in murine models of liver disease,[183] and the TLR4 inhibitor serelaxin (RLX030), when combined with the PPARγ agonist rosiglitazone, amplifies the beneficial effects of the latter compound.[221] In line with these findings, inhibition of the PAMP-responsive NLRP3 inflammasome with MCC950 also alleviates fibrosis in murine NASH models.[90] In addition, PAMP-dependent macrophage activation can be inhibited by elimination of the invasive microbiota; thus, the normal gut microbiome can be restored by broad-spectrum antibiotics, probiotics, and fecal microbiota transfer.[222,223]

Due to the overlap in inflammatory signal pathways (e.g., NF-κB, ASK1, JNK, and p38) between hepatic macrophages and hepatocytes, therapies targeting such pathways may affect both hepatocyte metabolism and macrophage activation.[224] One example includes the apoptosis signal-regulating kinase 1 (ASK-1) inhibitor selonsertib (GS-4997), which, in an early phase 2 trial, was shown to decrease the disease severity of NASH patients.[225] However, follow-up phase 3 trials including NASH patients with bridging fibrosis (NCT03053050) and cirrhosis (NCT03053063) failed to replicate these promising results.[226]

Shaping of macrophage function and polarization

Due to the duality of macrophage phenotype and thus function, therapies that induce a switch from the proinflammatory to regenerative phenotype would be beneficial for the treatment of liver disease. Such macrophage reprogramming can be principally achieved through different anti-inflammatory mediators, such as steroids (e.g., dexamethasone, a derivative of corticosterone), IL-4, IL-10, and PGE_2.[227]

Due to the high scavenging activity of KCs, the systemic administration of various drug delivery systems, such as liposomes and microbubbles, causes their accumulation in the liver,[228] highlighting their potential as macrophage-specific therapeutic approaches. For example, administration of dexamethasone-loaded liposomes ameliorates inflammation and fibrosis in murine models of inflammatory liver disease. Moreover, administration of dexamethasone-loaded vehicles results in significant inhibition of T-cell accumulation in the liver and a dominantly restorative (anti-inflammatory) macrophage phenotype.[229] It is speculated that through modification of drug carriers, such as through implementation of arginine-like ligands and the addition of mannose to the surface, distinct macrophage subsets could be targeted. However, such tailored drug-delivery systems have not yet advanced to clinical studies.[230,231]

Galectin-3, a β-galactoside-binding lectin predominantly expressed in macrophages, mediates important inflammatory functions and is known to exert profibrogenic effects in HSCs.[232] Although promising results have been achieved with the galectin-3 inhibitor GR-MD-02 in preclinical murine models,[233] it did not alleviate fibrosis in a phase 2 clinical trial in NASH patients.[234] The efficacy and safety of GB1211, another galectin-3 inhibitor, is currently under investigation (NCT03809052).

Peroxisome proliferator-activated receptors are nuclear transcription factors with distinct and multiple functions in NAFLD pathology, including effects on inflammation and lipid and glucose metabolism.[235] Moreover, in vitro administration of lanifibranor, a pan-PPAR agonist, significantly reduces inflammatory gene expression induced by stimulation of murine macrophages and patient-derived circulating monocytes with palmitic acid and even leads to enhanced expression of genes involved in lipid metabolism. The anti-inflammatory action of lanifibranor can be induced through agonism of PPARδ, as evidenced by the effects of individual PPAR agonists.[236] Moreover, lanifibranor treatment causes inhibition of MoMϕ accumulation, one of the key events preceding liver fibrosis, further highlighting its anti-inflammatory effects.[185] The beneficial effects of lanifibranor were shown in preclinical choline-deficient, amino acid-defined high-fat diet (CDAA-HFD)-fed mice and are thought to be the result of a combination of different modes of action that inhibit liver damage, inflammation, and HSC activation.[236] Lanifibranor is currently being investigated in a phase 2 clinical trial in NASH patients (NCT03008070).

In summary, intensive research in recent years has certainly improved our understanding of hepatic macrophages in the context of homeostasis and diseases. This rapid increase in knowledge related to the mechanisms of hepatic macrophages and the development of technical advances in targeted drug delivery have facilitated the translation of findings from rodent studies into novel therapies that can be used in the clinic.

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ADDITIONAL INFORMATION

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